



T-cell vaccination in multiple sclerosis

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Received 2 March 2003; accepted 4 May 2003

Abstract

T cells that are autoreactive against myelin antigens play a pivotal role in the pathogenesis of multiple sclerosis (MS). The concept of T cell vaccination (TCV) has been developed to generate an immune response against these autoreactive pathogenic T cells. Immunologic data accumulated so far demonstrates depletion of T cells reactive against immunodominant myelin peptides after immunization in the animal model of experimental autoimmune encephalomyelitis, as well as in vaccinated MS patients. Clinical trials have confirmed the safety and efficacy of TCV in a small number of immunized MS patients: TCV resulted in reduced relapse rates and slowed the progression of neurological disability and MRI brain lesion load. Recently, there have been several double-blind, placebo-controlled studies initiated to evaluate the role of TCV in MS. Specifically, it is important to examine the effect of early TCV, given after the first episode suggestive of the disease, in order to prevent the process of epitope spreading.

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Keywords: Multiple sclerosis; Autoimmunity; T-cell vaccination; Experimental autoimmune encephalomyelitis

1. Introduction

Multiple sclerosis (MS) is the most common inflammatory disease of the central nervous system (CNS) affecting young adults, and the third leading cause for neurological disability after trauma and arthritic disease [1,2]. The disease, which is more common in females (2:1), first appears between ages 20 and 40 with a peak onset of approximately 25 years. MS can present with different clinical courses; the most frequent is the relapsing-remitting (RR), which occurs in 85% of

patients. Fifty percent of patients with RR-MS will develop a secondary progressive (SP) course within 10 years from disease onset. Approximately 15% of patients, mainly males above the age of 40 years, present at onset with primary progressive (PP) disease. MS frequently causes motor, sensory, coordination, visual and/or cognitive impairment, as well as urinary or bowel dysfunction and symptoms of fatigue [3].

2. The immunological basis of MS

The etiology of MS is still an enigma. A variety of genetic, immunologic and environmental factors have been implicated in triggering its onset and

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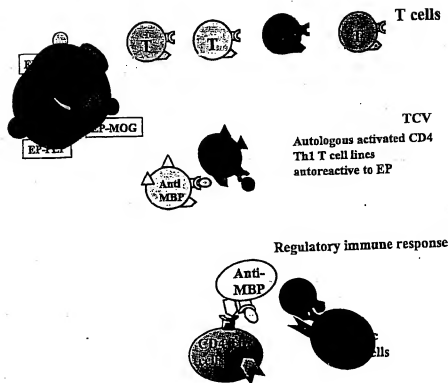


Fig. 1. Schematic representation of TCV.

progression [4,5]. Genetic background may play a role in the disease pathogenesis as MS is more common in Caucasians, and its frequency increases with distance from the Equator (in both hemispheres). Pathologically, MS is characterized by perivascular infiltration of monocytes and lymphocytes, mainly CD4⁺ T cells, within the brain and spinal cord that leads to myelin destruction [6]. The autoreactive T cells, which recognize a variety of self-antigens, represent part of the normal T cell repertoire and circulate naturally in the periphery without causing an autoimmune disease [7]. The critical transition from the normal physiological state of autoreactivity to autoimmune pathology is determined by the interplay between activation and clonal expansion of the autoreactive T cells, and the functioning of regulatory networks that keep them on track. In MS, autoreactive Th1 cells against myelin antigens like myelin basic

protein (MBP), myelin oligodendrocyte glycoprotein (MOG), or proteolipid protein (PLP) undergo in vivo activation and clonal expansion, infiltrate the blood brain barrier and lead to local inflammation with the production of pro-inflammatory cytokines (IL-2, TNF- α and γ -interferon), thus resulting in myelin-destructive inflammation in the CNS [8]. It has been shown that these autoreactive T cells can also induce experimental autoimmune encephalomyelitis (EAE), an animal model for MS [9].

3. Principles of T cell vaccination

Vaccination is a type of immune education in which exposure to an attenuated pathogen teaches the immune system to attack this pathogen in its virulent form. Most vaccinations against infectious agents are preventive, but some vaccinations, like rabies vaccination or tumor immunotherapy, can

be curative. The vaccine may be composed of the whole pathogen or of a constituent of it containing a vulnerable target antigen [10] (Fig. 1).

T cell vaccination (TCV), like any vaccination, activates a subject's immune system to neutralize a pathogenic agent. However, it differs from classical vaccination in that the object to be resisted is not a foreign infectious agent or tumor, but rather a population of the subject's own T cells; the vaccine is devised using a component of the immune system itself [11]. Although the biological cause of MS is unknown, the history of smallpox demonstrates that this knowledge is not essential for vaccination against the disease. In MS the myelin-attacking cells are considered as pathogens and are used for TCV. These cells are isolated from the peripheral blood, inactivated by irradiation, and injected as a vaccine to sensitize the body's immune system as to be able to recognize, and ultimately eliminate them from the blood.

4. Rationale of TCV in MS

TCV developed as an outgrowth of the studies initially performed by Cohen et al. [12] in the animal model of EAE, actively induced by autoimmunization of experimental animals with myelin antigens such as MBP or MOG. Immunization with irradiated T-cells, or T-cell lines sensitized against myelin antigens, was found to be effective in preventing the development of EAE [13,14]. A single inoculation with anti-MBP line was sufficient to protect approximately 70% of rats from subsequent actively induced EAE. The difference between inducing EAE and protection against EAE was dependent on the state of the administered T-cells; when attenuated by irradiation, the T-cells induced resistance, but when fully virulent the cells caused disease [15]. Moreover, to induce either disease or resistance the T-cells had to be activated by a myelin antigen. The resistance to EAE and the disease was prevented by T-cell lines directed against diverse antigenic determinants of MBP [16]. These studies in experimental animals paved the way for the first clinical trials aimed at evaluating TCV in MS patients.

5. TCV provisions

The following criteria are required for TCV to induce the desired immune response:

(1) *Two sets of signals*: Target antigen signals and accessory signals. The target antigen is the T cell receptor (TCR) or a part of it, which represents the autoreactive T cell responsible for the disease. The accessory signals required for effective TCV are activation markers (ergotypes). Autoreactive T cell lines/clones are generated during the TCV procedure by stimulation with several antigens related to MS, such as whole brain, myelin, or specific myelin peptides. However, autoreactive T cell clones can also be generated directly from the cerebrospinal fluid (CSF) without antigen stimulation [17].

(2) *Effectors*: The effector cells that respond to stimulation with the target antigen(s) are activated CD4⁺ cells of Th1 phenotype. The vaccination consists of these autoreactive cells and produces tissue-damaging Th1 cytokines such as IFN- γ and TNF- α [18,19].

(3) *Regulatory networks*: Vaccination induces inhibition of the effector cells by anti-idiotypic and anti-ergotypic responses. The specific TCR is the major target of both CD8 MHC class I restricted and CD4 class II restricted anti-idiotypic T cells. The anti-clonotypic T cell lines isolated from immunized patients are predominantly CD8 cytolytic cells that kill and eradicate the autoreactive effector cells involved in the disease [20]. Anti-clonotypic or anti-lineotypic CD4 cells are the major cytokine producing cells in the anti-vaccine cell population following TCV. The generation of regulatory CD4 Th2 cells by TCV induces an immunological shift from the pro-inflammatory Th1 to the anti-inflammatory Th2 activity by the production of anti-inflammatory cytokines like IL10 and IL4 [21]. Additionally, other cell populations expanded upon stimulation with the vaccine, including $\gamma\delta$ T cells and NK cells, may play a role in the TCV induced peripheral regulatory network [22]. Anti-ergotypic immune response directed against activation markers may play an important role in the suppression of the activated autoreactive T cells following vaccination. Although the target for these T–T cell

interactions remain unidentified, cytokine receptor CD25 has been proposed as a candidate molecule [23,24].

6. TCV clinical trials in MS

T cell vaccination has recently been studied as a potential treatment for patients with MS. These clinical studies are summarized in Table 1.

The first phase I clinical trial was reported by Hafler et al. [25] who vaccinated four patients suffering from progressive MS with CSF attenuated T cell clones that recognized immunodominant regions of myelin autoantigens and induced a partial short-term immunosuppression.

Subsequently, in a pilot trial Zhang et al. [20] reported depletion of circulating MBP-reactive T cells in six MS patients who were vaccinated with peripheral blood MBP reactive T-cell clones. The irradiated T cell clones were found to react against encephalitogenic MBP peptides (predominant response against peptides 84-102 and 143-168). The vaccinations resulted in beneficial clinical effects and were not associated with adverse events. Medaer et al. [26] reported that after vaccination with irradiated T cells reactive to MBP, five out of eight MS patients had decreased relapse rate and less increase in MRI lesion volume as compared with matched, untreated MS patients (8% vs. 39.5%, respectively). The trial was extended to 49 MS patients who were immunized with circulating MBP-reactive T cells and anti-clonotypic response against the vaccine cells was observed [26]. Two to five years after TCV, reappearance of MBP-reactive T cells was found in five out of nine vaccinated patients [27]. The new clone had a different origin from the clones isolated prior to vaccination and it was suggested that additional immunizations could result in their depletion. These results suggest that the autoimmune process in MS can be inhibited by TCV; the primary triggering mechanism, however, still continues to operate and induce the appearance of new autoreactive T cells.

In Weiner et al. [28], vaccinated four SP MS patients with bovine myelin-reactive irradiated T cell lines from the peripheral blood. Two patients evaluated by the expanded disability status scale

(EDSS) [29] showed stable neurological status. Over time, one patient showed improvement of one EDSS step while EDSS worsened in the other patient. A progressive decline of circulating whole myelin-reactive T cells, MBP143-168, PLP104-117, and MOG43-55-peptide-reactive T cells, was demonstrated following the second vaccination. All T cell lines lysed not only myelin-reactive T cells, but also T cell lines specific for MBP143-168, PLP104-117 and MOG43-55 peptides.

Zhang et al. [30] recently reported the results of TCV in 54 patients with RR-MS ($n=28$) or SP-MS ($n=26$) who were immunized with irradiated autologous MBP-reactive T cells. Depletion of MBP-reactive T cells correlated with a reduction by 40% in the rate of relapses in RR-MS patients as compared with the pre-treatment rate in the same cohort. However, the reduction in EDSS was minimal in RR-MS patients while the EDSS slightly increased in SP-MS patients over a period of 24 months. Serial semi-quantitative MRI examinations demonstrated stabilization in lesion activity as compared with baseline MRI.

Based on previous reports that have shown an accumulation of activated T cells recognizing multiple myelin antigens in the CSF of MS patients, Van der Aa et al. [17] conducted a pilot clinical trial using activated CD4⁺ T cells derived from the CSF as TCV in five MS patients (four RR, one PP). Three immunizations with irradiated CSF vaccines were administered over a 2-month interval. The vaccinations were well tolerated and no toxicity or adverse effects were reported. All patients remained clinically stable or had reduced EDSS with no relapses during or after the vaccination. The T cell lines showed reactivity to MBP, MOG and/or PLP and had a restricted clonality.

The findings accumulated so far suggest a potential clinical benefit for TCV in MS and encouraged us to evaluate TCV in non-responding MS patients. We generated T cell lines reactive against immunodominant peptides (MBP, MOG) in 20 RR-MS patients with an aggressive disease course who had failed to respond to various immunomodulatory treatments. In order to expand the scope of the vaccination process we applied a broader approach by the selection of T cell lines. The selection of clones during the vaccine preparation

Table 1
Clinical trials with TCV

Author	Patients	MS type	Origin of autoreactive T cells	Antigen	Interval between vaccination	Safety	Outcome
Hafler et al. [25]	4	PP	CSF				Partial short-term immunosuppression
Zhang et al. [20]	6	RR=3 PP=1 SP=2	PBMC clones	MBP-human brain	2 months; 3 vaccinations, SC; 1.5×10^6 cells/clone; 4 clones/vaccination	No side effects	Depletion of MBP autoreactive T cells
Medner et al. [26]	8	RR=5 PP=1 SP=2	PBMC clones	MBP		No side effects	Reduced relapse rate, reduced MRI lesion load as compared to matched controls
Hermans et al. [22] Correale et al. [28]	49 4	NA SP	PBMC clones PBMC by Leukopheresis; Lines	MBP Whole bovine myelin	3 months; 5–7 injections SC; 40×10^6 T cell lines	No side effects	EDSS, 2-stable; 1-worse; 1-better
Zhang et al. [30]	54	RR=28 SP=26	PBMC Clones	MBP protein and MBP immunodominant synthetic peptides 83–99; 151–170	2 months; 3 vaccinations, sc; $3-6 \times 10^6$ cells/vaccination; 2–4 clones/vaccination	No side effects	40% reduction in RR; stabilization by MRI and EDSS (RR>SP)
Van der Aa et al. [17]	5	RR=4 SP=1	CSF	No antigen	3 sc; Activated 10^6 CD4 T cells	No toxicity or adverse effects	No relapses during or after treatment; clinically stable or reduced EDSS
Achiron and Mandel (unpublished data)	20	RR	PBMC	MBP and MOG synthetic peptides	2 months; 3 vaccinations, SC; 1.5×10^6 cells/line; up to 5 lines/vaccination	No toxic effect or adverse events	Reduction in relapse rate, decreased rate of progression to disability, stabilization MRI

might narrow the anti-clonotypic immune responses to the immunizing clones only, leaving unregulated a large portion of the autoreactive T-cell population that exist in this polyclonal disease. Patients were immunized with 3 vaccinations in 6–8 week intervals and were monitored for changes in rate of relapse, neurological disability as measured by the EDSS score and MRI lesion load for a period of 15 months. No serious adverse events were observed. A reduced relapse rate and stabilization of neurological disability were observed when compared to the 1-year pre-treatment rate. Automated computerized quantitative MRI analysis demonstrated significant reduction in the number and volume of active lesions, as well as reduction in T2 lesion burden (Achiron and Mandel, unpublished data).

7. On-going TCV clinical trials in MS

(1) In 1999 the group lead by L. Weiner in LA, USA initiated a double-blind, placebo-controlled Phase II clinical trial involving 80 patients with secondary-progressive MS. Half of the participants received TCV against whole bovine myelin and the other half received inactive placebo. Eleven sc vaccinations with 40 million lymphocytes (1 ml) will be repeated over a 2-year course, and patients will be clinically monitored for safety and efficacy.

(2) In March 2002, the group lead by Zhang, Killian, and Rivera, at Baylor College School of Medicine, Houston, Tx, USA began an open label study of multiple TCVs in 18 RR-MS patients for a period of 24 months. Outcome parameters include duration of MS relapses, frequency of relapse, EDSS and MRI.

(3) In September 2000, the group led by Medaer, Stinissen, and Raus (Dr Willems-Instituut, Diepenbeek, Belgium) began a double-blind, placebo-controlled study involving 60 RR-MS patients who received 3 sc vaccinations of 50×10^6 myelin-specific attenuated T cells derived from the CSF. Outcome parameters include MRI studies performed bimonthly for a period of 18 months.

(4) In Spring 2002, Karussis, Abulafia and Abramsky (Hadassah Hospital, Jerusalem, Israel)

began a double-blind study with TCV in 30 RR-MS patients for a 1-year period.

(5) In September 2002, our group (MS center & Blood Bank, Sheba Medical Center, Tel-Hashomer, Israel) initiated a double-blind, randomized, placebo-controlled trial in 76 patients with probable MS. Half of the patients receive 3 sc vaccinations of attenuated T-cell lines reactive to immunodominant MBP, PLP and MOG synthetic peptides, and the other half receive placebo injections. This study aimed to evaluate the effect of TCV in patients after the first neurological episode suggestive of MS. It is of utmost importance to assess whether early treatment with TCV will prevent the second attack-conversion to definite MS. Moreover, at disease onset, the immunological process of epitope spreading associated with the immune system's exposure to myelin antigens is still limited. With additional attacks there is increased recognition of new self-determinants of the encephalitogenic peptides presented to the immune system during the inflammatory process which enhances further disease activity. The aim of early treatment is to stop this process as early as possible, at disease onset, and thus to prevent its progression. Outcome parameters include rate of conversion to definite MS, time to second relapse, EDSS and MRI lesion load.

8. Summary

Clinical trials in MS will provide important information regarding the efficacy of TCV as a therapeutic procedure for the disease. It appears that effective vaccination will be dependent on skewing the immune response in such a way that it is not harmful to the host. It will be of value, therefore, to optimize the vaccine composition, target antigens, the number of T cells within the vaccine and the schedule of vaccinations, as well as evaluate the long-term effects of TCV. Furthermore, it is necessary to develop immunologic parameters to follow each vaccinated patient's particular state in order to make decisions about the need of additional vaccinations in regard to epitope spreading. Finally, assuming that TCV proves efficient, it will be necessary to scale up

and automate the procedure to enable cost-effective vaccinations for large MS populations.

Take-home messages

- Patients with MS have circulating autoreactive T cells that react against myelin peptides.
- Immunization with attenuated autoreactive T-cell lines/clones, using the patient's own activated T cells specifically to target antigens is a procedure termed T-cell vaccination.
- The vaccine cells stimulate regulatory networks that induce direct depletion of the host pathogenic T cells by CD8 cytotoxic cells and initiate CD4 Th2 anti-inflammatory activity.
- TCV prevented the development of EAE in laboratory animals.
- Clinical trials in MS patients demonstrated reduced rates of clinical relapse, and slowed progression of disability and disease activity, which correlated with stabilization of the brain lesion load in brain MRI examinations.
- Future double-blind, placebo-controlled trials will shed light on the efficacy of TCV early in the disease process, as well as on the attempt to cure autoimmune disease by generating regulatory immune networks.

References

- [1] Kremenchutzky M, Cottrell D, Rice G, et al. The natural history of MS: a geographically based study. 7. Progressive-relapsing and relapsing-progressive MS: a re-evaluation. *Brain* 1999;122(Pt 10):1941–50.
- [2] Wallin MT, Page WF, Kurtzke JR. Epidemiology of MS in US veterans. VIII. Long-term survival after onset of MS. *Brain* 2000;123(Pt 8):1677–87.
- [3] Achiron A, Barak Y. Multiple sclerosis—from probable to definite diagnosis: a 7-year prospective study. *Arch Neurol* 2000;57(7):974–9.
- [4] Barcellos LF, Oksenberg JR, Green AJ, et al. Genetic basis for clinical expression in MS. *Brain* 2002;125(Pt 1):150–8.
- [5] Compston A, Sawcer S. Genetic analysis of MS. *Curr Neurol Neurosci Rep* 2002;2(3):259–66.
- [6] Stinissen P, Raus J. Autoreactive T lymphocytes in MS: pathogenic role and therapeutic targeting. *Acta Neurol Belg* 1999;99(1):65–9.
- [7] Zhang J, Raus J. T cell vaccination in MS: hopes and facts. *Acta Neurol Belg* 1994;94(2):112–5.
- [8] Sharief MK, Hentges R. Association between tumor necrosis factor- α and disease progression in patients with MS. *N Engl J Med* 1991;325(7):467–72.
- [9] Ben Nun A, Wekerle H, Cohen IR. The rapid isolation of clonable antigen-specific T lymphocyte lines capable of mediating autoimmune encephalomyelitis. *Eur J Immunol* 1981;11(3):195–9.
- [10] Cohen IR. T-cell vaccination for autoimmune disease: a panorama. *Vaccine* 2001;20(5–6):706–10.
- [11] Cohen IR, Weiner HL. T-cell vaccination. *Immunol Today* 1988;9(11):332–5.
- [12] Lider O, Reshef T, Beraud E, Ben Nun A, Cohen IR. Anti-idiotypic network induced by T cell vaccination against EAE. *Science* 1988;239(4836):181–3.
- [13] Ben Nun A, Cohen IR. Vaccination against autoimmune encephalomyelitis (EAE): attenuated autoimmune T lymphocytes confer resistance to induction of active EAE but not to EAE mediated by the intact T lymphocyte line. *Eur J Immunol* 1981;11(11):949–52.
- [14] Ben Nun A, Lando Z. Detection of autoimmune cells proliferating to Myelin basic protein and selection of T cell lines that mediate EAE in mice. *J Immunol* 1983;130(3):1205–9.
- [15] Cohen IR. Vaccination against experimental autoimmune disease using autoimmune T-lymphocytes. *Isr J Med Sci* 1988;24(12):734–6.
- [16] Holoshitz J, Frenkel A, Ben Nun A, Cohen IR. Autoimmune encephalomyelitis (EAE) mediated or prevented by T-lymphocyte lines directed against diverse antigenic determinants of MBP. Vaccination is determinant specific. *J Immunol* 1983;131(6):2810–3.
- [17] Van Der AA, Hellings N, Medaer R, et al. T cell vaccination in MS patients with autologous CSF-derived activated T cells: results from a pilot study. *Clin Exp Immunol* 2003;131(1):155–68.
- [18] Kumar V, Sercarz E. Distinct levels of regulation in organ-specific autoimmune diseases. *Life Sci* 1999;65(15):1523–30.
- [19] Liblau RS, Singer SM, McDevitt HO. Th1 and Th2 CD4+ T cells in the pathogenesis of organ-specific autoimmune diseases. *Immunol Today* 1995;16(1):34–8.
- [20] Zhang J, Medaer R, Stinissen P, Hafler D, Raus J. MHC-restricted depletion of human MBP-reactive T cells by T cell vaccination. *Science* 1993;261(5127):1451–4.
- [21] Zang YC, Hong J, Tejada S, et al. Th2 immune regulation induced by T cell vaccination in patients with MS. *Eur J Immunol* 2000;30(3):908–13.
- [22] Hermans G, Denzer U, Lohse A, Raus J, Stinissen P. Cellular and humoral immune responses against autoreactive T cells in MS patients after T-cell vaccination. *J Autoimmun* 1999;13(2):233–46.
- [23] Kuniyasu Y, Takahashi T, Itoh M, Shimizu J, Toda G, Sakaguchi S. Naturally anergic and suppressive CD25(+) , CD4(+) T cells as a functionally and

- phenotypically distinct immunoregulatory T cell sub-population. *Int Immunol* 2000;12(8):1145–55.
- [24] Takahashi T, Tagami T, Yamazaki S, et al. Immunologic self-tolerance maintained by CD25(+)CD4(+) regulatory T cells constitutively expressing cytotoxic T lymphocyte-associated antigen 4. *J Exp Med* 2000;192(2):303–10.
- [25] Hafler DA, Cohen I, Benjamin DS, Weiner HL. T cell vaccination in multiple sclerosis: a preliminary report. *Clin Immunol Immunopathol* 1992;62(3):307–13.
- [26] Medaer R, Stinissen P, Truyen L, Raus J, Zhang J. Depletion of myelin-basic-protein autoreactive T cells by T-cell vaccination: pilot trial in multiple sclerosis. *Lancet* 1995;346(8978):807–8.
- [27] Hermans G, Medaer R, Raus J, Stinissen P. Myelin reactive T cells after T cell vaccination in MS: cytokine profile and depletion by additional immunizations. *J Neuroimmunol* 2000;102(1):79–84.
- [28] Correale J, Lund B, McMillan M, Ko DY, McCarthy K, Weiner LP. T cell vaccination in SP MS. *J Neuroimmunol* 2000;107(2):130–9.
- [29] Kurtzke JR. Rating neurologic impairment in MS: an expanded disability status scale (EDSS). *Neurology* 1983;33(11):1444–52.
- [30] Zhang JZ, Rivera VM, Tejada S, et al. T cell vaccination in MS: results of a preliminary study. *J Neurol* 2002;249(2):212–8.

The World of Autoimmunity; Literature and Synopsis

Mannose-binding lectin autoantibodies in SLE

Mannose-binding lectin (MBL) has structural similarities to C1q. Anti-C1q antibodies can be detected in Mannose-binding lectin (MBL) has structural similarities to C1q. Anti-C1q antibodies can be detected in patients having systemic lupus erythematosus (SLE), and hence Seelen et al. (*Clin Exp Immunol* 2003;134:335) tested the prevalence of anti-MBL antibodies among these patients. They found that the levels of anti-MBL antibodies were significantly higher among SLE patients compared with controls. However, no difference in anti-MBL levels were found between patients with active and inactive disease. Furthermore, as opposed to anti-C1q autoantibodies, the occurrence of anti-MBL were not associated with renal disease. Nonetheless, a significant correlation was found between anti-C1q and anti-MBL autoantibodies. Anti-MBL influenced the functional activity of MBL as there was a negative correlation between the levels of anti-MBL antibodies and MBL-complex.

Anti-p53 antibodies in lung diseases

The altered p53 gene in some cancers can lead to the production of anti-p53 autoantibodies. Neri et al. (*Lung Cancer* 2003;39:165) compared the frequency of these antibodies in several groups of patients with lung diseases. Anti-p53 autoantibodies were not detected in healthy controls whereas they have been found in 2 of 30 (6.7%) patients having pleural malignant mesothelioma, and 8 of 48 (16.7%) patients having lung cancer. No correlation was found regarding age, sex, cancer stage or histology, cigarette smoking or occupational exposure. This study emphasizes that the minority of patients with lung cancer have anti-p53 autoantibodies. It is unclear yet whether this immune response is associated with a better outcome (such as longer survival as a trend towards it was found in this study), a worse prognosis, or no association with disease course.

Phenotype and genotype findings in SLE patients with thrombocytopenia

Systemic lupus erythematosus (SLE) is a multi-systemic autoimmune disease characterized by diverse clinical manifestations. Of note is that 2 patients having SLE might have complete different clinical picture. Scofield et al. (*Blood* 2003;101:992) studied 38 pedigrees of SLE patients having thrombocytopenia in order to find out whether this manifestation of SLE (which predicts a severe disease) is associated with specific genetic markers and clinical manifestations in patients' families. Linkages were established at 1q22–23 in the 38 pedigrees and at 11p13 in the 13 African American pedigrees. The clinical manifestation found in association with thrombocytopenia were nephritis, serositis, anti-dsDNA antibodies, autoimmune hemolytic anemia, neuropsychiatric SLE, and antiphospholipid antibodies. Families of a patient having thrombocytopenia had a more severe SLE. These findings further support the high mortality associated with thrombocytopenia in SLE. Future similar studies could help to sub-classify SLE into different types based on clinical manifestation, genetic markers or both.

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